THE OCCURRENCE AND COMPOSITION OF MANNA IN EUCALYPTUS AND ANGOPHORA

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Synopsis

Manna, an exudate from the injured leaves and branches of certain eucalypts and angophoras, has a different composition from the sap. The mode of occurrence, composition and the methods of analysis employed are given. An hypothesis is advanced that the manna is the result of the action of the enzymes of the saliva of insects on the sugars present in the phloem sap.

INTRODUCTION

The secretion of manna by certain eucalypts was noted early in the 19th century. Virey (1832) described it in a paper to the *Journal de Pharmacie* and Mudie mentioned it in a report on *E. mannifera* in 1834. In 1843 Johnston examined this manna and distinguished it from the manna of commerce. The principal sugar in eucalyptus manna he called melitose (now called raffinose) whereas commercial manna consists mainly of mannitol. A saccharine exudation from *E. punctata* was examined by Smith (1897) who identified the sugar with raffinose. He observed that the manna exuded from a wound in the tree was caused by the larva of a wood-borer. It was noted that where the puncture had not entirely penetrated the bark, the exudation was white and attracted ants, but where the puncture had penetrated right through the bark and had entered the wood, a mixture of manna and kino exuded and was not taken by ants. Smith found that the manna consisted of raffinose and a small amount of reducing sugar, that it was exuded from the tree as a syrupy liquid and crystallized on evaporation. No further reference to eucalyptus manna has been found except a statement in several books that *E. viminalis* secretes a sugary substance called manna.

In the present paper the term " manna " will be confined to the saccharine secretion from the trees themselves. It excludes the sugary secretion from aphids, scale, lerps and other insects. These have been called manna by some writers, e.g. Penfold and Willis (1961), but are entirely different in composition from the manna discussed in this paper.

MODE OF OCCURRENCE AND COMPOSITION

Manna has been observed not only on the wood of *E. punctata* and on the leaves of *E. viminalis* and *E. mannifera* as mentioned above, but the writer has collected it also from the wood of *E. maculata* and from the leaves of *E. punctata*, *E. maculata*, *E. citriodora*, *E. tereticornis*, *Angophora floribunda* and *A. costata*.

On leaves and twigs it occurs as white nodules of interlaced acicular crystals, the nodules varying in diameter from 1·5 to 4 mm. and weighing about 0·005 to 0·03 grammes. Occasionally larger nodules occur but, after growing to a certain size, most of the nodules appear to be dislodged by the motion of the leaves in the wind or they are dissolved by dew or rain. The largest nodule found on a leaf was the size of a pea and weighed 0·07 gramme. The secretion from the trunk or a large branch of a tree may weigh several grammes, but it generally consists of a number of smaller nodules or tears.

The manna occurs only on the site of a wound inflicted by an insect. In more than 600 attempts to obtain manna by wounds artificially inflicted by punching holes in leaves, scarifying twigs, cutting leaves in half, drilling a hole in the trunk or cutting a blaze in the tree, not one resulted in the generation of manna. On the other hand, leaves partly eaten by an insect or timber infested with a borer will frequently produce manna from the wound. The secretion of manna does not take place immediately a leaf is injured. Several hours or even days may elapse before exudation commences. In the case of a leaf the manna always occurs at the severed end of a vein (Fig. 1). On a terminal shoot the nodule of manna conforms to the shape of the phloem sheath of the shoot and sometimes invests the truncated end of the shoot. The manna is secreted from the vein of a leaf at the rate of 0·001 to 0·0025 gramme per day. There is evidence that in some cases the rate of growth of a nodule varies,

Fig. 1. Leaf of *E. maculata* showing nodules of manna on end of veins.

the nodule being alternately large and small in diameter. When a nodule is removed from a leaf, the wound often continues to " bleed " and form another nodule. As the manna exudes from the wound it crystallizes below that already formed. The nodules thus increase by accretion from below and, not like a stalactite, by having the newer material deposited on the distal end. The manna is secreted from the leaves and young twigs as a liquid but appears to crystallize immediately. No free liquid has been observed in the nodules on the leaves. The secretion from the trunk and large branches of a tree, however, often remains liquid for some time and may run down the trunk several centimetres before it crystallizes.

The secretion of manna takes place throughout the year but appears to be most abundant in the spring and early summer, when the growth of new leaves is most rapid. Also, it has been noticed that the nodules occur more frequently on the side of the tree facing the sun. The rate of formation of manna thus appears to be related to the rate of flow of the sap.

The analysis of several specimens of manna from the leaves and twigs of angophoras and eucalypts has shown that it is not just dried sap which has exuded from the wound. The comparison of paper chromatograms of the sugars of manna and of the juice expressed from an uninjured leaf of the same age as that from which the manna was taken, shows a marked qualitative and quantitative difference in composition. The approximate proportions of the various sugars present in the manna and the corresponding leaves are shown in Table 1.

Actually, in most cases of injury to a leaf or to a branch the wound does not "bleed". Only about one of every hundred injured leaves will secrete manna. It appears that some special environment or condition is needed to permit the formation of manna. It is postulated that this condition is the secretion of an enzyme by the insect, possibly in the saliva, which hydrolyses the pectins and hemicelluloses of the cells forming, *inter alia*, galactose and/or galactose phosphate which, under the influence of another galactosidase or glycosidase, synthesizes raffinose from sucrose, melibiose from glucose and, in some cases, stachyose from raffinose present in the phloem fluid. It may be suggested that the causative agent is not the saliva of the insect but a micro-organism introduced indirectly into the wound. That this is improbable is supported by the observation of Fisher (1945) that inoculation of cuts on *Myoporum platycarpum* with three micro-organisms most abundant in its manna did not cause manna formation.

TABLE 1

Comparison of the approximate proportions of sugars in the manna with those of the leaf-sap of E. maculata, E. punctata *and* A. costata

Sugar	*E. maculata*		*E. punctata*		*A. costata*	
	Manna	Leaf	Manna	Leaf	Manna	Leaf
Stachyose	0	0	2	0	10	0
Raffinose	80	5	80	10	65	10
Melibiose	10	0	0	0	0	0
Sucrose	6	85	10	80	20	70
Glucose and Fructose ..	4	10	8	10	5	20

The presence of galactose in the tissue surrounding the injury to leaves has been detected, but no galactose has been found in the uninjured part of the leaf. That this process of synthesis of raffinose is possible is supported by another investigation (not yet complete) in which the sap of the stems of *E. maculata* is ingested by a scale insect, *Eriococcus coriaceus* (Mask.). The secretion of this insect consists of a mixture of some five or six galactosides varying in complexity from di- to penta-saccharides. In this case the cell sap has actually passed through the alimentary tract of the insect as distinct from the manna which is formed in the plant substance itself. It is suggested that some similar enzyme is involved in both cases. It is relevant to this investigation to note that Lechevallier (1962) obtained an α-galactosidase from germinating barley with which she converted sucrose into raffinose.

Since the concentration of sugars in the phloem sap is not constant, being dependent on the rate of photosynthesis, translocation, metabolic transformations and other processes, so the composition of manna varies. In the leaf, which is relatively rich in sucrose and poor in glucose, the manna consists largely of raffinose and contains little or no melibiose. The phloem sap of the trunk and larger branches, on the other hand, contains a much higher proportion of glucose and consequently the manna is richer in melibiose. This sugar is about 20 times as soluble in water as raffinose and hence the exudate from the trunk and larger branches takes longer to crystallize and is more fluid than that of the leaves. This accounts for its habit of often running down the trunk or branch several centimetres before it crystallizes.

The results of the analyses of specimens of manna from different sources are shown in Table 2. It will be seen that they vary slightly from sample to sample but in general they consist of about 60% sugars, 16% water (mainly water of crystallization), and a small amount of ash. The remaining 20% has been shown to contain pectin and uronic acids. The manna has a pH of 5·3 and an acid number of 3·75 to 5·1

It should be noted that mannitol, a major constituent of the manna of *Myoporum platycarpum* (Hatt and Hillis, 1947), has not been found in any specimen of eucalyptus or angophora manna so far examined.

The individual sugars and their relative proportions in each specimen of manna were determined by paper chromatography. The identity of the sugars was deduced by comparison of their spots on the chromatogram with those of authentic specimens of the respective sugars when subjected to certain tests. These tests included their $R_{glucose}$ values, the characteristic colours given with various spray reagents, the preparation and examination of the osazones (of those sugars which yielded them) and, in the case of tri- and tetra-saccharides, by identification of the products of hydrolysis with acid and with invertase.

TABLE 2

The composition of specimens of manna from different sources

	E. maculata Leaf	*E. punctata* Leaf	*E. punctata* Trunk
Loss on drying	16·6	14·4	17·04
Ash	3·6	1·3	1·6
P_2O_5	0·66	Trace	Trace
Total sugars (as glucose) ..	58·8	66·0	61·17
Reducing sugars (as glucose) ..	4·3	4·2	18·72
	79·0	81·7	79·81

It is not always possible to determine which insect inflicted a particular wound from which manna exudes. The secretion of manna does not take place immediately an insect attacks the plant, so that frequently the insect causing the wound has gone before exudation commences. However, some of the less mobile insects have been observed on the site of the manna secretion. For example, the blister on a leaf caused by the leaf-miner *Philactophaga eucalypti* (Frogg.) has been found with a number of nodules of manna attached to the margin inside the blister. The larva of the leaf case-moth *Hyalarcta hubneri* (Wwd) and of the saw-fly *Perga dorsalis* (Leach) have both been identified as causing the secretion of manna. A wound in the trunk of *E. punctata* from which manna was flowing was caused by the larva of a beetle (not identified).

The majority of the specimens of manna, both eucalypt and angophora, came from the leaves of suckers growing around the stumps of trees that had been felled. Only rarely do specimens occur on mature trees. The most abundant source of leaf manna is *E. maculata*, followed by *E. mannifera* and *E. punctata*. Only very few specimens have been taken from the leaves of *E. citriodora*, *E. tereticornis* and the two angophoras. None of the other eucalypts in this district has yielded any specimens. The most productive source of manna from the trunk and large branches is *E. punctata*.

EXPERIMENTAL

The sugars present in the manna were identified by paper chromatography and the results confirmed by other tests such as the examination of the osazones and the products of hydrolysis by acid and by invertase. In the chromatographic examination, Whatman's no. 1 paper was used. The descending solvent method was found to give the best results, using as developing solvents A, butanol-acetone-water 3 : 4 : 1 and, when a two dimensional chromatogram was required, solvent B, butanol : ethyl acetate : acetic acid : water 5 : 4 : 2 : 2.

As reference sugars, stachyose, raffinose, sucrose and glucose were mainly used, but other standard sugars were used on occasions. Table 3 shows the $R_{glucose}$ values of the sugars for solvents A and B at 25°.

After about 20 hours' development in the tank the papers were dried at 105° and treated by the method of Bailey and Bourne (1960). The components of the manna were identified not only by their $R_{glu.}$ value but also by the characteristic colours given by the spray. The presence of the fructofuranose group in the molecule was detected by spraying with naphthoresorcinol : HCl : acetone 1 : 2 : 200. The group was indicated by the development of a reddish-orange colour on heating.

Table 3

$R_{glucose}$ values of sugars at 25° for solvents A and B

Sugar	Solvent A	Solvent B
Stachyose	0·06	0·058
Raffinose	0·24	0·18
Melibiose	0·33	0·26
Sucrose	0·66	0·56
Galactose	0·86	0·90
Glucose	1·00	1·00
Fructose	1·15	1·29

The oligo-saccharides were further identified by hydrolysis with 0·2N HCl and also with invertase. Hydrolysis by HCl was carried out at 70° for one hour and hydrolysis by invertase at 35° for 24 hours.

The determination of the relative proportions of stachyose to raffinose, of raffinose to sucrose, etc., was made by comparing the area and intensity of colour of the stains on a chromatogram with those of a number of standard solutions of sugars of known concentration.

References

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